Stem Nematode and Northern Root-Knot Nematode Resistance Ratings for Alfalfa Cultivars and Experimental Lines

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ABSTRACT

The stem nematode (Ditylenchus dipsaci (Kuhn) Filipjev) and the northern root-knot nematode (Meloidogyne hapla Chitwood) are important pests of alfalfa (Medicago sativa L.). Little information is available characterizing the levels of resistance in existing cultivars. This publication characterizes 179 domestic and foreign alfalfa cultivars and 9 experimental lines for stem and northern root-knot nematode resistance. Results indicated that most cultivars are susceptible to the two nematodes; however, good levels of resistance were exhibited in some cultivars and in the experimental check lines. It was evident that resistance to both the stem and root-knot nematode could be obtained through plant breeding efforts.

KEYWORDS: Alfalfa, Ditylenchus dipsaci, Medicago sativa, Meloidogyne hapla, nematode resistance, root-knot nematode, stem nematode.

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STEM NEMATODE AND NORTHERN ROOT-KNOT NEMATODE RESISTANCE RATINGS FOR ALFALFA CULTIVARS AND EXPERIMENTAL LINES

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The stem nematode (Ditylenchus dipsaci (Kühn) Filipjev) and the northern root-knot nematode (Meloidogyne hapla Chitwood) are important pests of alfalfa (Medicago sativa L.) (7) 2/. Stem nematodes are destructive pests primarily in irrigated regions of the United States (2); however, they are found occasionally in nonirrigated areas (fig. 1). They feed and reproduce in the lower part of the stems and in crown buds. Infested buds become swollen and distorted and fail to elongate into normal stems, resulting in significant yield reduction.

Northern root-knot nematodes are found in most areas in the northern two-thirds of the United States where alfalfa is grown (9), although they are causing significant problems only in scattered areas (fig. 2). They feed and reproduce in the roots of alfalfa, causing small galls that resemble nodules. Severe stand and vigor reduction can result from heavy infections.

Losses due to nematodes, primarily stem and root-knot nematodes, have been estimated at 3 to 5 percent of the national alfalfa production (9) but may prove to be higher. Alfalfa breeders have found that nematode resistance can be increased by selection procedures. Breeders in the Western United States are currently developing locally adapted, high-yielding alfalfa germplasm carrying good resistance to both the stem and root-knot nematodes. Information on the relative levels of resistance in existing cultivars and experimental lines would be very useful to them. The farmer selecting a cultivar to plant may also find such information helpful. This publication reports levels of stem nematode and northern root-knot nematode resistance identified in 179 domestic and foreign cultivars and 9 experimental check lines in tests at Prosser, Wash., in 1972 and Reno, Nev., in 1978. With five exceptions--Chimo, Deseret, Joaquin 11, Orchies, and Titan--all alfalfa cultivars approved by the Alfalfa Variety Review Board through 1977 were tested.

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2/ Underlined numbers in parentheses refer to Literature Cited at the end of this report.

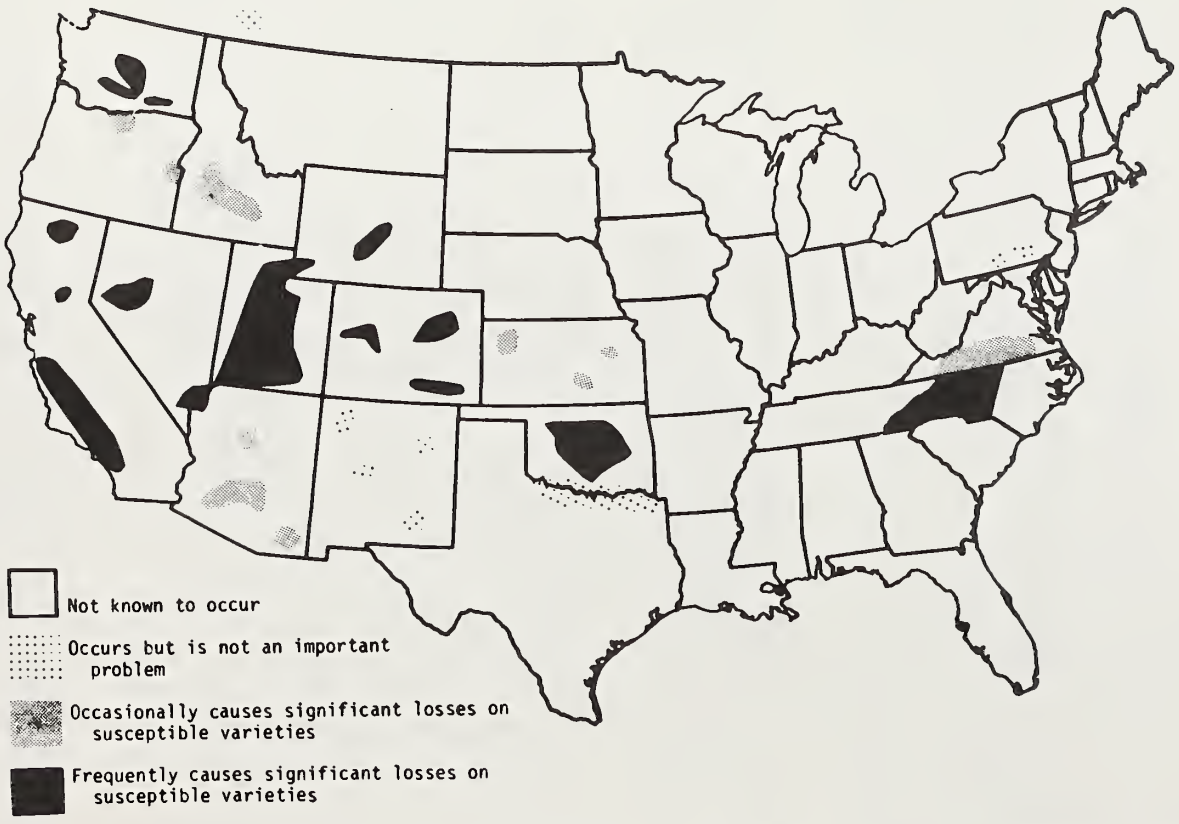

Figure 1.--Stem nematode (Ditylenchus dipsaci (Kuhn) Filipjev) distribution and severity on alfalfa in the United States.

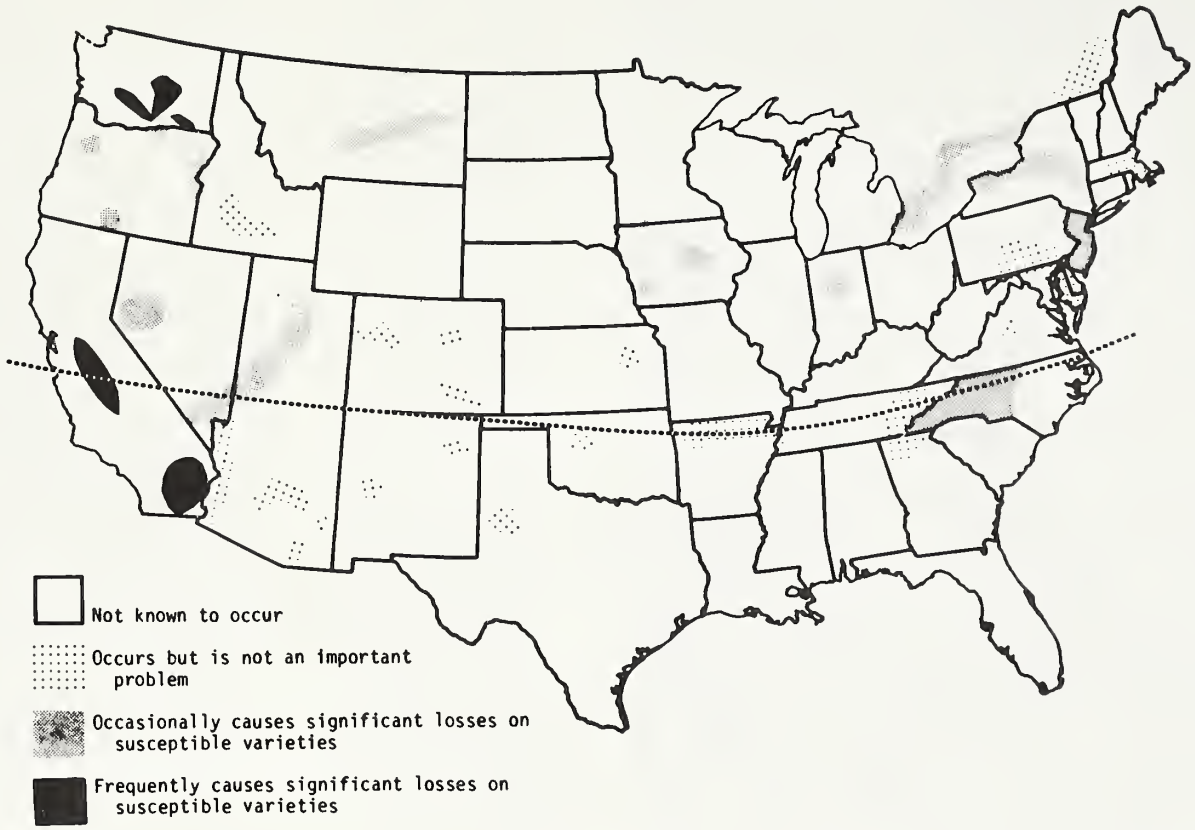

Figure 2.--Root-knot nematode (Meloidogyne sp.) distribution and severity on alfalfa (southern species below line, northern species above line).

EVALUATION PROCEDURES

Stem Nematode

For the entries evaluated at Prosser, alfalfa seeds were germinated on moist filter paper in petri dishes to insure uniform seedling emergence. They were planted in rows (10 seeds per row, 2 rows per entry) in metal flats (31 by 51 cm) filled with steam-pasteurized, very fine, sandy loam soil. Each flat contained 12 rows. Five replications were used. Seeds were covered with soil, and the flats were inoculated with an aqueous suspension of stem nematodes at the rate of 50 nematodes per seed. The flats were maintained in the greenhouse at about 20° C with a 12-hour-average natural photoperiod.

Nematodes used for this study were cultured on alfalfa callus tissue grown on a nutrient medium (6). They were extracted from the callus tissue by placing the infected plant tissue and medium on a 20-mesh sieve screen covered with 2-ply facial tissue. The screen was set in a pan of water so the water barely covered the plant tissue and the nematodes settled in the water below the screen. The water with the nematodes was collected daily and was stored in a refrigerator at 3° C until used. Most of the nematodes were extracted within 48 hours. (Nematodes should be used as soon as possible after extraction to avoid lowering their infectivity.) They settle about 5 cm per hour in standing water and could therefore be concentrated by siphoning off the upper water after 2 to 4 hours. Nematode numbers were determined by suspending the nematodes in a given volume of water, counting them in three or four 1-ml samples using a stereomicroscope, and multiplying by the milliliter volume of the suspension.

Stand counts were made 2 weeks after planting, and the seedlings were inoculated a second time with a nematode suspension at the rate of 200 nematodes per seedling. To insure against escapes, third and fourth inoculations of 200 nematodes per seedling were made at 4 and 6 weeks. Plant tops were clipped at 10 weeks. At 16 weeks after planting, the tops were clipped and the numbers of symptomless plants (those without swollen, distorted stems and buds) were counted for each entry (fig. 3). Percentages of stem-nematode-resistant plants were calculated for each entry as follows:

$$\text{Percent stem-nematode-resistant plants} = \frac{\text{Number of symptomless plants at 16 weeks}}{\text{Number of plants present in stand at 2 weeks}} \times 100$$

Additional details on this and other stem nematode screening procedures may be found in earlier publications (2-4).

For entries evaluated at Reno, alfalfa seeds were germinated on moist blotter paper and transplanted to fine washed sand. Unlike the Prosser procedure, seedlings were inoculated at about 3 weeks (first trifoliolate stage) by atomizing an aqueous suspension containing 120 larvae per milliliter. Approximately 50 ml were applied per flat of 300 seedlings. Moist cheesecloth was placed over the seedlings and this was covered with plastic sheeting. After 24-hour incubation, a second inoculation similar

Figure 3.--Stem nematode damage on 16-week-old alfalfa plants inoculated at 2 weeks of age: Left, severe symptoms (susceptible); middle, moderate symptoms (susceptible); right, no symptoms (resistant).

to the first was applied. Plants were again covered and incubated an additional 24 hours. They were evaluated for stem nematode symptoms 10 weeks after planting.

Root-Knot Nematode

For entries evaluated at Prosser, 20 germinating seeds of each entry were planted per row in soil-filled flats similar to those used for the stem nematode evaluation. Each entry was planted in a single row and five replications were used. The seeds were covered with soil and the flats were maintained in the greenhouse at about 25° C with a 12-hour-average natural photoperiod. Two weeks after planting, the flats were inoculated with an aqueous suspension of active root-knot nematodes at the rate of 400 nematodes per seedling. A second inoculation of 250 nematodes per seedling was made 5 weeks after planting. Plant tops were clipped when flower buds began to form. Plants are usually rated at 12 to 16 weeks for root galling caused by the nematode. However, because of evidence of only a light infection, a third inoculation was made 16 weeks after planting with 600 active root-knot nematodes per seedling.

Nematodes for this study were cultured on roots of tomato plants in pots in the greenhouse. The tomato roots were prepared for nematode extraction by cutting and discarding the plant tops, washing the infected roots with water, and disinfecting the root surface in 10 percent Clorox solution for 5 minutes. Nematode extraction was performed by placing the clean roots on a 20-mesh sieve screen covered with 2-ply facial tissue and setting the screen in a pan of water with the water level slightly above the screen surface. The roots were sprinkled with captan fungicide to inhibit fungus growth and covered with moist facial tissue.

Newly hatched larvae began emerging in less than 24 hours, settled into the water below the screen, and continued emerging for 7 to 21 days. The water was changed daily, and the nematodes were stored in a refrigerator at 3° C until they were used. (Nematodes should be used as soon as possible after extraction to avoid lowering their infectivity.) As with the stem nematode, root-knot nematodes settle about 5 cm per hour in standing water and could therefore be concentrated by siphoning off the upper water after 2 to 4 hours. Nematode numbers were determined by suspending the nematodes in a given volume of water, counting them in several 1-ml samples using a stereomicroscope, and multiplying by the milliliter volume of the suspension.

At 24 weeks after planting, all plants were dug and examined for the presence of root galls (fig. 4). Those plants having one or more galls were considered susceptible. Most susceptible plants had more than 50 galls. Those plants with no galls were classified as resistant. Percentages of resistant plants examined were calculated.

Additional details on this and other root-knot nematode screening procedures may be found in earlier publications (1, 3).

For entries evaluated at Reno, seeds were pregerminated and planted in flats of nematode-infested sand that was prepared by mixing sand in which infected tomato plants were grown with clean washed sand (1:1). Plants were evaluated 12 weeks after planting using the same procedure as at Prosser.

RESULTS AND CONCLUSIONS

Levels of resistance to the stem and root-knot nematodes are presented in table 1. Stem nematode resistance ranged from 0 percent for four cultivars to 81 percent for the Washington experimental check W2S1 and 90 and 86 percent for Apalachee, tested at Prosser and Reno, respectively. Apalachee is a stem-nematode-resistant cultivar released by the former Agricultural Research Service (ARS) 3/ and cooperating State agencies in 1971. Most cultivars tested were susceptible; however, 21 cultivars and 6 experimental check lines exhibited resistance at 50 percent or higher. Among these lines were the Washington-resistant experimentals WDS3, W1S1, and W2S1. WDS3, W1S1, and cycle 2 (W2S2) of W2S1 were released as germplasm to alfalfa breeders by ARS and the Washington Agricultural Experiment Station in 1976 (5). Among the resistant cultivars were Washoe and Lahontan, both of which

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3/ Now part of the Science and Education Administration.

Figure 4.--Severe northern root-knot nematode galling on a susceptible 16-week-old alfalfa plant.

have been widely used in the stem nematode problem areas of the Western United States. Most of the other stem-nematode-resistant cultivars, such as Talent, Apalachee, and Nematol II, are highly susceptible to bacterial wilt and thus have received little interest in the problem areas. However, they may be of considerable benefit to breeders as a source of stem nematode resistance in new cultivar development.

Root-knot nematode resistance ranged from 0 percent for 14 cultivars to 100 and 92 percent for the experimental check Nev. Syn XX tested at Prosser and Reno, respectively. Most cultivars were considered susceptible. Only four cultivars had 50 percent or higher resistant plants. These cultivars trace to African germplasm where good resistance has been demonstrated. All five root-knot nematode-resistant experimental checks included in the study demonstrated a high level of resistance. Nev. Syn XX, which also has relatively high resistance to the stem nematode, and Nev. Syn YY were released as germplasm to alfalfa breeders by ARS and cooperating State agencies in 1974 (10) and 1978 (8), respectively.

Table 1.--Levels of stem and root-knot nematode resistance for selected domestic and foreign cultivars and experimental check lines 1/

Entry	Stem nematode		Root-knot nematode	
	Plants tested	Resistance	Plants tested	Resistance
Cultivar	Number	Percent	Number	Percent
A-24	72	40	33	3
A-57	227	10 *	136	5 *
A-59	83	11	64	16
African	83	2	59	54
Agate	87	13	131	5 *
Alfa	75	51	42	2
Algonguin	---	---	158	5 *
Altfranken	91	34	25	0
Amador	205	24 *	120	5 *
Anchor	94	20	37	3
Angus	---	---	106	5 *
Apalachee (Prosser)	86	90	28	4
Apalachee (Reno)	151	86 *	---	---
Apex	96	50	35	3
Apollo	203	19 *	113	7 *
Aragon	87	78	45	2
Arc	148	12 *	121	7 *
Ardiente	164	13 *	122	11 *
Arnim	92	13	26	0
AS-13	66	24	59	24
AS-13R	158	41 *	108	17 *
AS-49	91	33	42	5
AS-49R	168	38 *	127	4 *
Atlantic	90	14	59	8
Atlas	180	19 *	133	4 *
Atra 55	73	3	37	3
Baker	241	14 *	116	4 *
Beaver	84	0	47	2
Blazer	140	51 *	130	3 *
Bonanza	86	7	54	30
Bonus	155	11 *	113	9 *
Buffalo	74	15	45	2
Caliente	94	0	66	52
Caliverde	92	11	64	16

See footnote at end of table.

Table 1.--Levels of stem and root-knot nematode resistance for selected domestic and foreign cultivars and experimental check lines 1/--Con.

Entry	Stem nematode		Root-knot nematode	
	Plants tested	Resistance	Plants tested	Resistance
Cultivar	Number	Percent	Number	Percent
Caliverde 65.....	68	28	47	11
Cardinal.....	54	57	37	3
Cayuga.....	86	10	47	2
Cherokee.....	91	27	51	2
Citation.....	204	14 *	103	12 *
Conquest.....	212	21 *	115	8 *
Cossack.....	86	24	42	2
CUF 101.....	173	15 *	158	15 *
Culver.....	90	12	40	5
Dachenfeldt Isis.....	---	---	13	0
Dawson.....	85	12	70	1
Delta.....	85	19	46	2
Drylander.....	113	10 *	106	4 *
DuPuits.....	71	49	44	7
El-Unico.....	71	6	52	19
Emeraude.....	95	61	34	9
Europe.....	72	61	45	0
Eynsford.....	55	42	21	0
FD 100.....	78	53	44	2
Florida 66.....	62	8	78	29
Fremont.....	67	12	63	13
G 777.....	86	43	---	---
Glacier.....	87	32	44	0
Gladiator.....	88	34	126	4 *
Grimm.....	94	4	40	0
Hayden.....	93	4	18	11
Haymor.....	96	29	37	5
Honeoye.....	197	12 *	111	3 *
Iroquois.....	86	16	50	12
Isis.....	86	33	48	2
Kane.....	167	7 *	89	2 *
Kanza.....	91	12	65	2
Kayseri.....	90	50	30	0
Ladak.....	68	6	34	0

See footnote at end of table.

Table 1.--Levels of stem and root-knot nematode resistance for selected domestic and foreign cultivars and experimental check lines 1/--Con.

Entry	Stem nematode		Root-knot nematode	
	Plants tested	Resistance	Plants tested	Resistance
Cultivar	Number	Percent	Number	Percent
Ladak 65	91	13	42	0
Lahontan (Prosser)	81	53	38	3
Lahontan (Reno)	177	48 *	---	---
Lew	81	31	45	9
Liberty	289	20 *	154	6 *
Marathon	302	32 *	119	4 *
Mark II	67	4	48	27
Matador	133	12 *	99	9 *
Mediterranea	73	3	26	4
Mesa-Sirsa	81	5	41	2
Mesilla	73	44	58	24
Moapa	71	3	81	32
Moapa 69	86	1	62	34
Monsefu	78	0	84	23
Mustang	---	---	8	13
N-74	88	64	38	5
Narragansett	90	22	64	33
Nemastan	61	59	31	6
Nematol II	62	74	48	6
Nomad	92	10	59	2
Norseman	94	7	26	4
Nugget	211	51 *	145	3 *
Olympic	186	21 *	121	14 *
Orestan	75	8	33	6
Pacer	201	9 *	108	6 *
Peak	107	55 *	132	3 *
Peruvian	90	8	56	20
Phytor	150	14 *	132	4 *
Polar I	327	36 *	115	9 *
Polesana	70	1	29	0
Progress	100	12	45	18
Rambler	83	8	42	7
Rambler A	40	13	33	3
Ramsey	83	5	140	2 *

See footnote at end of table.

Table 1.--Levels of stem and root-knot nematode resistance for selected domestic and foreign cultivars and experimental check lines 1/--Con.

Entry	Stem nematode		Root-knot nematode	
	Plants tested	Resistance	Plants tested	Resistance
Cultivar	Number	Percent	Number	Percent
Ranger	92	8	60	2
Resistador	77	44	33	6
Rhizoma	82	2	78	31
Riley	153	8 *	130	3 *
Romer	73	16	42	7
San Isidro	96	50	50	10
Saranac	98	30	57	0
Saranac AR	201	17 *	124	2 *
Scout	95	11	58	19
Sevelra	76	14	52	2
Sirsa #9	96	11	51	16
Slavenskaya	60	18	36	11
Socheville	97	45	30	7
Sonora	95	3	66	50
Sonora 70	83	2	71	23
Spredor	222	14 *	70	19 *
Stride	84	37	25	4
SX 10	216	10 *	114	2 *
Talent	89	73	61	7
Team	70	46	57	18
Tempo	92	16	43	2
Teton	95	5	44	14
Thor	88	30	56	5
Tierra de Campos	99	45	31	10
Travois	89	9	39	23
Triesdorfer	88	36	43	0
Tuna	81	48	50	0
UC Cargo	204	34 *	132	17 *
UC Salton	82	7	98	14 *
Uinta	54	2	35	9
Valor	155	16 *	146	10 *
Vanguard	175	10 *	99	8 *
Vernal	96	20	56	36
Victoria	75	3	53	8

See footnote at end of table.

Table 1.--Levels of stem and root-knot nematode resistance for selected domestic and foreign cultivars and experimental check lines 1/--Con.

Entry	Stem nematode		Root-knot nematode	
	Plants tested	Resistance	Plants tested	Resistance
Cultivar	Number	Percent	Number	Percent
Vista	203	18 *	170	3 *
Warrior	89	16	45	18
Washoe	78	54	43	14
Weevlchek	88	32	71	8
Williamsburg	96	19	56	5
WL 202	51	18	52	6
WL 210	94	14	52	48
WL 214	95	11	40	20
WL 215	88	10	65	26
WL 216	89	15	118	5 *
WL 219	213	6 *	114	6 *
WL 220	163	13 *	158	9 *
WL 303	94	40	59	20
WL 305	93	16	68	46
WL 306	96	21	69	22
WL 307	91	21	127	13 *
WL 308	92	33	148	8 *
WL 309	90	33	119	6 *
WL 310	204	44 *	125	6 *
WL 311	181	19 *	141	5 *
WL 318	209	20 *	172	4 *
WL 450	89	29	173	16 *
WL 451	89	35	119	9 *
WL 501-R	93	27	133	17 *
WL 504	84	12	48	31
WL 508	85	15	82	38
WL 512	224	7 *	53	20 *
WL 600	66	24	---	---
Zia	90	44	54	11
123	94	2	52	38
131	192	18 *	69	20 *
153	94	1	69	1
167	85	56	144	8 *
183	86	0	86	67

See footnote at end of table.

Table 1.--Levels of stem and root-knot nematode resistance for selected domestic and foreign cultivars and experimental check lines 1/--Con.

Entry	Stem nematode		Root-knot nematode	
	Plants tested	Resistance	Plants tested	Resistance
Cultivar	Number	Percent	Number	Percent
185	84	12	62	48
520	91	2	30	13
521	175	13 *	133	12 *
522	87	7	63	25
524	215	10 *	152	3 *
525	64	16	58	33
530	96	20	130	3 *
531	206	18 *	102	7 *
545	211	24 *	154	4 *
572	204	11 *	155	8 *
581	258	53 *	160	8 *
Experimental check line				
WDS3	95	75	---	---
W1S1	94	72	---	---
W2S1	89	81	---	---
W12S1	89	75	---	---
W14R1	---	---	61	92
W9SR1	91	64	68	75
Nev. Syn WW	---	---	68	75
Nev. Syn XX (Prosser)	82	54	62	100
Nev. Syn XX (Reno)	---	---	116	92 *
Nev. Syn YY	---	---	122	91 *
LSD (0.05) Prosser	---	14	---	17
LSD (0.05) Reno.	---	23	---	16

1/ Values without * were derived from 1972 evaluations at Prosser, Wash., and those with * were derived from 1978 evaluations at Reno, Nev. Dashes indicate data not available.

It is clearly evident from our evaluations that the level of resistance to both the stem and root-knot nematodes can be markedly increased through plant breeding efforts. The improved resistance in the experimental entries in table 1 substantiates this conclusion. With continuing plant breeding efforts, high levels of stem and root-knot nematode resistance should become standard characteristics of alfalfa cultivars in the future.

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